Identification of new OPA1 cleavage site reveals that short isoforms regulate mitochondrial fusion

Ruohan Wang, Prashant Mishra, Spiros Garbis, Annie Moradian, Michael Sweredoski, and David Chan

Corresponding author(s): David Chan, California Institute of Technology

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Review Timeline:

Submission Date:	2020-09-22
Editorial Decision:	2020-11-06
Revision Received:	2020-11-08
Accepted:	2020-11-11

Editor-in-Chief: Matthew Welch

Transaction Report:

RE: Manuscript #E20-09-0605
TITLE: "Identification of new OPA1 cleavage site reveals that short isoforms regulate mitochondrial fusion"

Dear Dr. Chan:

Your manuscript entitled "Identification of new OPA1 cleavage site reveals that short isoforms regulate mitochondrial fusion" has been read by two expert referees. They both report that the experimental evidence and interpretations are solid. They also agree that the paper does little to advance our mechanistic understanding of how the long and short isoforms of OPA1 together function in mitochondrial fusion. However, they disagree diametrically on whether the paper should be published in MBoC, as opposed to a more specialized journal, as you will see when reading their reports.

My own reading of the paper leads me to favor the opinion of referee #1, supporting publication, based on the notion that this is a solid step towards a better understanding of mechanism.

#1 points out that your paper should cite and discuss the work of Ge, et al. published earlier this year in eLife. Both referees raise questions that you may wish to speculate about in the Discussion.

With your revised manuscript, please send us a point by point summary of your responses to the referees' comments, and the revisions you make to the paper.

Thank you for submitting this interesting work to Molecular Biology of the Cell.

Sincerely,
Thomas Fox
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chan,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The focus of this study is on how long and short isoforms of the mitochondrial inner membrane fusion protein OPA1 are generated in cells. It is know that a combination of these isoforms are required for efficient fusion, which was recently directly demonstrated in an vitro reconstituted system (eLife). Here, the authors convincingly demonstrate that OPA1 exon4b encodes a previously unknown additional cleavage site for the the protease, YME1L (termed S3). S3 was uncovered by experiments showing that deletion of the known YMEL1 S2 cleavage site did not lead to any mitochondrial or physiological phenotypes at the cellular or organismal level. Through the characterization of S3, the work also reinforces the importance of a mixture of long and short OPA1 isoforms for efficient fusion. Although the work does not advance our understanding at a mechanistic level of the relative roles of OPA1 long and short isoforms in fusion, it advances what we know about of the roles and interplay of YMEL1 and OMA1 cleavage site in generating short isoforms in cells.

In discussing the roles of s- and l- OPA1, the authors should include the observation from the Ge et al eLife manuscript showing that while a mixture of l- and s-OPA1 is required for efficient fusion, relatively high levels of s- relative to l-OPA1 can also inhibit fusion, which likely explains why cells are fragmented upon s-OPA1 overexpression.

Can the authors speculate why S3 is more efficiently cleaved than S2 by YMEL1 at a mechanistic level?

Is the tissue specificity of splice variants and their relative abundance known? Is this regulated?

Reviewer #2 (Remarks to the Author):

In this manuscript Wang and colleagues describe the discovery of a new proteolytic cleavage site, S3, in the OPA1 GTPase. OPA1 mediates fusion of mitochondrial inner membranes, and it remains controversial whether the uncleaved long form is responsible for fusion on its own, or together with the cleaved short form. The authors show that constitutive processing to the short form relies on the S3 cleavage site, and that inner membrane fusion relies on the stoichiometry of long to short OPA1 forms.

The data presented in this paper is of high quality and its interpretation is sound. This work certainly represents a step closer towards understanding the maintenance of mitochondrial morphology. It does, however, not contain any mechanistic insight into how the long and short forms of OPA1 might contribute to mitochondrial fusion (does length of the protein affect GTPase activity, GTP binding, binding to interaction partners, oligomerization?) and is therefore better suited for a more specialized journal.

Dear Dr. Fox,

Thank you for handling our manuscript.

You indicate that “#1 points out that your paper should cite and discuss the work of Ge, et al. published earlier this year in eLife. Both referees raise questions that you may wish to speculate about in the Discussion.“ As indicated in the point-by-point responses below, we have now cited the Ge et al paper and revised the text to address the issues raised by the reviewers. All text changes are indicated in red in the revised manuscript.

Reviewer #1 (Remarks to the Author):

The focus of this study is on how long and short isoforms of the mitochondrial inner membrane fusion protein OPA1 are generated in cells. It is know that a combination of these isoforms are required for efficient fusion, which was recently directly demonstrated in an vitro reconstituted system (eLife). Here, the authors convincingly demonstrate that OPA1 exon4b encodes a previously unknown additional cleavage site for the the protease, YME1L (termed S3). S3 was uncovered by experiments showing that deletion of the known YMEL1 S2 cleavage site did not lead to any mitochondrial or physiological phenotypes at the cellular or organismal level. Through the characterization of S3, the work also reinforces the importance of a mixture of long and short OPA1 isoforms for efficient fusion. Although the work does not advance our understanding at a mechanistic level of the relative roles of OPA1 long and short isoforms in fusion, it advances what we know about of the roles and interplay of YMEL1 and OMA1 cleavage site in generating short isoforms in cells.

In discussing the roles of s- and l- OPA1, the authors should include the observation from the Ge et al eLife manuscript showing that while a mixture of l- and s-OPA1 is required for efficient fusion, relatively high levels of s- relative to l-OPA1 can also inhibit fusion, which likely explains why cells are fragmented upon s-OPA1 overexpression.

Response: This is an excellent point. We now cite the Ge et al paper in the Introduction and also the Discussion. In the revised manuscript, text changes are indicated in red.

Can the authors speculate why S3 is more efficiently cleaved than S2 by YMEL1 at a mechanistic level?

Response: This is an interesting question. In the revised manuscript, the following text in the Discussion addresses this issue:

> In this study, we have identified a new site, S3, that is efficiently cleaved by Yme1L to yield s-OPA1. It remains to be determined why YME1L cleaves at S3 much more efficiently than at S2. A short hydrophobic sequence (FAWFP) has been found to act as a degron for YME1L (26). However, no similar sequences are apparent in exon 4b or the rest of OPA1. Upon recognition of substrates, YME1L uses ATP hydrolysis to progressively translocate them towards the proteolytic center (27, 28). It is plausible that S3, or the region surrounding it, increases the recognition of OPA1 as a Yme1L substrate or its translocation. Future studies can address these issues.

Is the tissue specificity of splice variants and their relative abundance known? Is this regulated?

Response: Yes, the splice variants are differentially expressed in tissues (both human and mouse), and these studies are now cited in the Introduction. We do not know of any study on regulation of splice variant expression.

Reviewer #2 (Remarks to the Author):

In this manuscript Wang and colleagues describe the discovery of a new proteolytic cleavage site, S3, in the OPA1 GTPase. OPA1 mediates fusion of mitochondrial inner membranes, and it remains controversial whether the uncleaved long form is responsible for fusion on its own, or together with the cleaved short form. The authors show that constitutive processing to the short form relies on the S3 cleavage site, and that inner membrane fusion relies on the stoichiometry of long to short OPA1 forms.

The data presented in this paper is of high quality and its interpretation is sound. This work certainly represents a step closer towards understanding the maintenance of mitochondrial morphology. It does, however, not contain any mechanistic insight into how the long and short forms of OPA1 might contribute to mitochondrial fusion (does length of the protein affect GTPase activity, GTP binding, binding to interaction partners, oligomerization?) and is therefore better suited for a more specialized journal.

Response: Our study focuses on the biological impact of having long and short OPA1 isoforms. It shows that the ratio of long to short isoform is critical for mitochondrial fusion. This was an issue that had been disputed. Having established this concept, future studies can target the biochemical basis for this effect.

RE: Manuscript #E20-09-0605R
TITLE: "Identification of new OPA1 cleavage site reveals that short isoforms regulate mitochondrial fusion"

Dear Dr. Chan:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Thomas Fox
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Chan:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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Sincerely,

Eric Baker
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